Mechanism-Based Inactivation of tRNA-Guanine Transglycosylase from *Escherichia coli* by 2-Amino-5-(fluoromethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one†

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ABSTRACT: In *Escherichia coli*, tRNA-guanine transglycosylase (TGT) catalyzes the incorporation of the queuine precursor preQ$_1$ [2-amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one] into tRNA. This precursor is further elaborated to queuine by two subsequent enzymic reactions [Slany, R. K., & Kersten, H. (1994) *Biochimie 76*, 1178−1182]. Our previous studies [Hoops, G. C., Townsend, L. B., & Garcia, G. A. (1995) *Biochemistry* (in press)] on a series of synthetic 5- and 6-substituted 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones have revealed that the *E. coli* TGT tolerates a wide diversity of substituents (isosteric, or nearly so, to the aminomethyl group of preQ$_1$) at the 5 position. We report here that 2-amino-5-(fluoromethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (FMPP) inactivates TGT in a time- and concentration-dependent manner with k_{inact} = 0.074 min^{-1} and K_I = 136 μM. A competitive inhibitor (7-methyl-preQ$_1$), with respect to preQ$_1$, of TGT [Hoops, G. C., Townsend, L. B., & Garcia, G. A. (1995) *Biochemistry* (in press)] protects the enzyme from inactivation by FMPP. FMPP also acts as a competitive inhibitor (K_I = 114 μM) of TGT under initial velocity conditions. The rate of fluoride release from FMPP is slightly faster (0.064 min^{-1}) than the k_{inact} (0.053 min^{-1}) at 300 μM FMPP, consistent with fluoride release preceding inactivation. FMPP appears to partition between "normal" turnover (k_{cat} = 0.461 min^{-1} and K_m = 152 μM), inactivation, and an alternative processing to an unidentified, fluoride-released product. This inhibitor (FMPP), if it possesses suitable physical properties for cellular uptake, may serve as a useful biological probe in determining the role of the queuine modification in tRNA as well as an *in vitro* mechanistic probe for the *E. coli* TGT.

tRNA-guanine transglycosylase (TGT,[1] EC 2.4.2.29) catalyzes the incorporation of the hypermodified base queuine [2-amino-5-[[(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl)amino]-methyl]pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one, Figure 1] into queuine-cognate tRNAs (tRNA^{Asp}, tRNA^{Asn}, tRNA^{His}, and tRNA^{Tyr}) (Okada & Nishimura, 1979). In *Escherichia coli*, TGT catalyzes the incorporation of the queuine precursor preQ$_1$ [2-amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one, Figure 1] into tRNA. This precursor is further elaborated to queuine by two subsequent enzymic reactions (Slany & Kersten, 1994). We have discovered that the TGT from *E. coli* is a zinc metalloprotein (Chong *et al.*, 1995), and some of the aspects of tRNA recognition by the *E. coli* TGT have been elucidated (Curnow & Garcia, 1994, 1995; Curnow *et al.*, 1993; Mueller & Slany, 1995; Nakanishi *et al.*, 1994). Studies on a series of synthetic 5- and 6-substituted 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones have revealed that the *E. coli* TGT tolerates a wide diversity of substituents (isosteric, or nearly so, to the aminomethyl group of preQ$_1$) at the 5 position and that 7-methylated analogues are competitive inhibitors, with respect to guanine, of TGT-catalyzed exchange of radiolabeled guanine into tRNA (Hoops *et al.*, 1995).

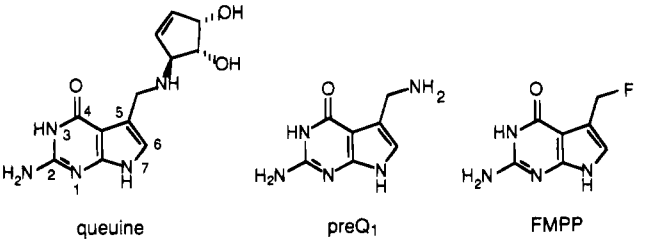

FIGURE 1: Queuine, preQ$_1$, and 2-amino-5-(fluoromethyl)pyrrolo-[2,3-*d*]pyrimidin-4(3*H*)-one (FMPP).

Very little is known about the mechanism of the TGT reaction. Serine 90 has been shown to be involved in catalysis by TGT (Reuter *et al.*, 1994), but its exact role is unknown. Substrate and product analyses clearly indicate that deprotonation of the pyrrole ring nitrogen of preQ$_1$ (N-7, Figure 1) must occur prior to the subsequent glycosidic bond formation. Recognizing this fact, we have designed a potential mechanism-based inactivator (suicide substrate) for TGT that involves the enzyme-catalyzed formation of a Michael acceptor in the TGT active site. We report here that 2-amino-5-(fluoromethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (FMPP, Figure 1) is a mechanism-based inactivator of TGT.

MATERIALS AND METHODS

Reagents. Buffers and reagents were purchased from Sigma. [8-^{14}C]Guanine (56 mCi per millimole) was from

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[1] Abbreviations: TGT, tRNA-guanine transglycosylase; preQ$_1$, 2-amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one; FMPP, 2-amino-5-(fluoromethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TCA, trichloroacetic acid; TLC, thin-layer chromatography.

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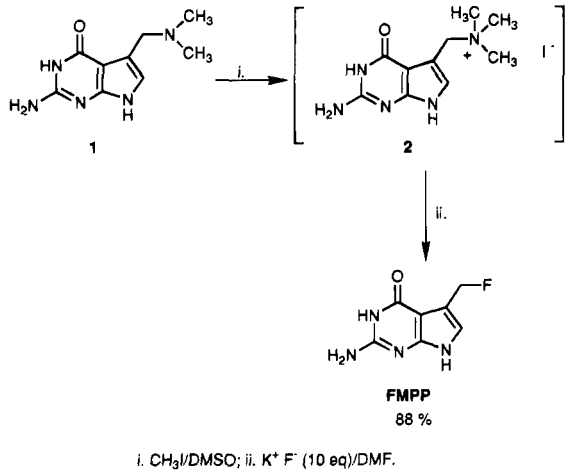

FIGURE 2: Synthetic Scheme for FMPP.

Moravek Biochemicals. Wild-type *E. coli* TGT was isolated from an overexpressing clone as previously described (Chong & Garcia, 1994; Garcia *et al.*, 1993). Fully unmodified *E. coli* $tRNA^{Tyr}$ was prepared as previously described (Curnow & Garcia, 1994; Curnow *et al.*, 1993). A 10 mL transcription reaction mixture consistently yielded 2.5 mg of tRNA after purification. This tRNA was stored at −20 °C as an ethanol precipitate.

2-Amino-5-(fluoromethyl)pyrrolo[2,3-d]pyrimidin-4(3H)-one (FMPP). While a variety of synthetic routes to FMPP were explored, a one-pot reaction sequence, shown in Figure 2, was chosen. 2-Amino-5-[(*N*,*N*-dimethylamino)methyl]-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**1**) was prepared via a modification of a literature procedure (Akimoto *et al.*, 1988). 2-Amino-5-[(*N*,*N*-dimethylamino)methyl]pyrrolo[2,3-*d*]-pyrimidin-4(3*H*)-one (**1**) (100 mg, 0.48 mmol) was dissolved in dimethyl sulfoxide (5 mL). Methyl iodide (0.5 mmol) was added and the solution stirred at 25 °C for 0.5 h. The solution was then diluted into dimethylformamide (45 mL), and potassium fluoride (5.0 mmol) was added. The mixture was stirred at 50 °C for 12 h. The solvent was removed *in vacuo* (aspirator) and the residue washed with H_2O (2 × 5 mL). The crude product was purified by silica gel column chromatography in EtOAc/EtOH/acetone/H_2O (20:2:2:1) (v:v:v:v): yield 77 mg (88%); mp 286 °C dec; R_f [in $CHCl_3$/MeOH (9:1) (v:v)] 0.04; R_f [in EtOAc/EtOH/acetone/H_2O (20:2:2:1) (v:v:v:v)] 0.17; ^{1}H-NMR (DMSO-d_6) δ 4.82 (d, J = 32 Hz, 2H, CH_2F), 5.9 (br s, 1H, 2-NH_2), 6.35 (d, J = 3.1 Hz, 2H, H-6), 10.1 (br s, 1H, NH), 10.5 (br s, 1H, NH). Anal. Calcd for $C_7H_7N_4OF$: C, 46.16; H, 3.87; N, 30.76. Found: C, 46.18; H, 3.95; N, 30.67.

Inactivation of TGT by FMPP. TGT (5 μM) was incubated at 37 °C with FMPP (0−300 μM), tRNA (20 μM), and HEPES assay buffer (100 mM HEPES (pH 7.3), 20 mM $MgCl_2$, and 5 mM DTT). Aliquots were removed at 5 min intervals (3 min for higher inhibitor concentrations) and diluted 50-fold into HEPES assay buffer containing 10 μM [8-^{14}C]guanine and 20 μM tRNA in a total volume of 400 μL. The enzyme was then assayed for activity at 37 °C by removal of 75 μL aliquots at regular intervals and precipitation of the tRNA in 2 mL of 5% TCA. The precipitated tRNA was then collected by filtration on GF/C filters and washed twice with TCA and once with ethanol. The radioactivity was quantitated by liquid scintillation counting and converted to picomoles of guanine incorporated. The percent activity remaining was calculated relative to a control experiment containing no inhibitor in the preincubation mixture. In all analyses, it was assumed that there is only one active site per enzyme monomer and all of the enzyme was active.

Irreversibility of Inactivation. Totally inactivated TGT was prepared by incubation of TGT (5 μM) with FMPP (100 μM) and tRNA (20 μM) in HEPES assay buffer (with 20 mM DTT) at 37 °C for 8 h. A TGT activity assay was performed as described above to ensure complete inactivation. The inactivated TGT was separated from unbound FMPP and tRNA by anion exchange chromatography (Pharmacia Mono Q HR 10/10) in a gradient of 0.1 to 0.5 M NaCl in 25 mM HEPES (pH 7.3) and 5 mM DTT. The repurified enzyme was concentrated to 1.0 μM, and a 40 μL aliquot was assayed for activity as described above.

Determination of K_I for Competitive Inhibition of TGT by FMPP. FMPP was examined as an inhibitor of TGT-catalyzed incorporation of [8-^{14}C]guanine into tRNA under initial velocity conditions. Assay mixtures of 400 μL included 1 μM TGT, 10−200 μM FMPP, 20 μM tRNA, and 5−25 μM [8-^{14}C]guanine in HEPES assay buffer. Aliquots (75 μL) were precipitated, filtered, and quantitated as described above.

Protection from Irreversible Inactivation of TGT by a Competitive Inhibitor. TGT (5 μM) was incubated at 37 °C with 100 μM FMPP and 7-methyl-preQ$_1$ [2-amino-5-(aminomethyl)-7-methylpyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one, (Hoops *et al.*, 1995)] (0.5 or 20 μM) in HEPES assay buffer containing 20 μM tRNA. The enzyme was assayed for activity by removal of aliquots at regular intervals and 40-fold dilution into HEPES assay buffer containing saturating tRNA (20 μM) and [8-^{14}C]guanine (10 μM). Aliquots (75 μL) were precipitated, filtered, and quantitated as described above.

TGT-Catalyzed Elimination of Fluoride Ion from FMPP. The reaction of FMPP with TGT was analyzed for elimination of fluoride ion at 37 °C with an Orion Model 96-09 combination fluoride electrode with an Orion pH/millivolt meter. The electrode was calibrated using various dilutions of fluoride standard solution (Orion no. 940906) in HEPES assay buffer. Near the limit of detection (1 μM), a nonlinear but reproducible relationship between [F^-] and millivolts was noted. Experimental values were corrected by nonlinear computer fit to the calibration curve. The reaction solution contained 20 μM TGT, 300 μM FMPP, and 40 μM tRNA in HEPES assay buffer.

"Normal" Turnover of FMPP by TGT. FMPP was examined as a substrate for TGT-catalyzed incorporation into tRNA utilizing a previously described [8-^{14}C]guanine wash-out assay (Okada *et al.*, 1979). tRNA is prelabeled with [8-^{14}C]guanine (G*-tRNA) via TGT-catalyzed guanine exchange and reisolation (via ethanol precipitation) of the labeled tRNA. The incorporation of FMPP is followed by monitoring the release of radiolabel from the G*-tRNA. Assay mixtures of 400 μL included 50 nM TGT, 10−300 μM FMPP, and 20 μM G*-tRNA in HEPES assay buffer. Aliquots (75 μL) were precipitated, filtered, and quantitated as described above.

RESULTS

Inactivation of TGT by FMPP. Preliminary experiments indicated that FMPP (200 μM) caused complete inactivation

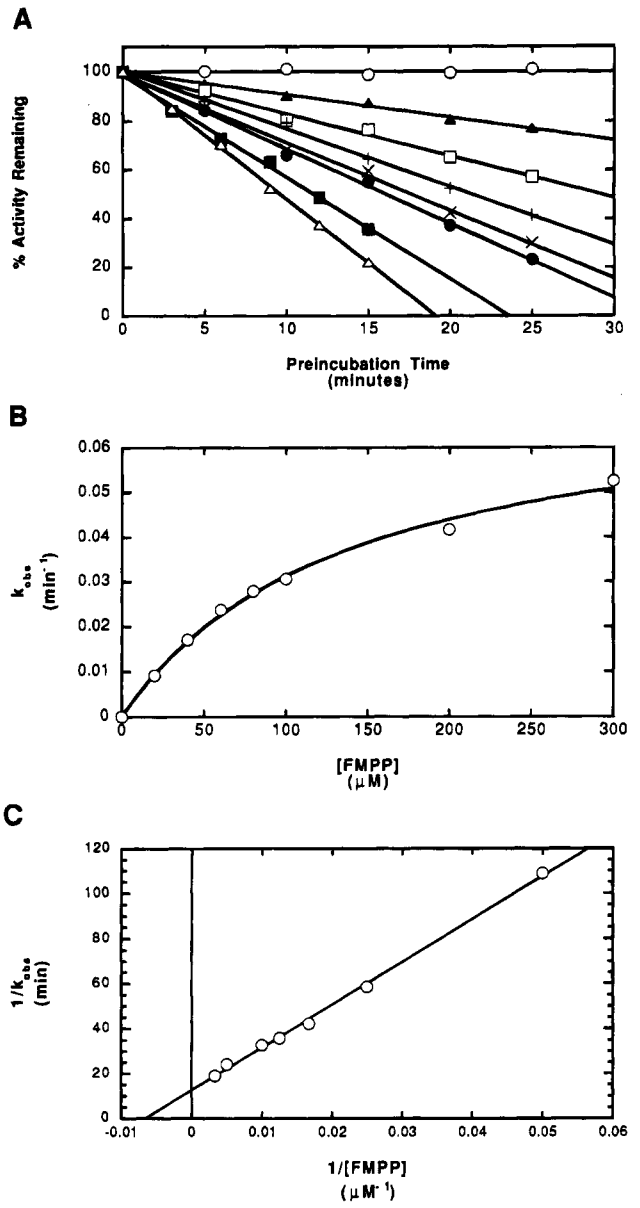

FIGURE 3: Inactivation of TGT by FMPP. (A) Time and concentration dependence of inactivation. The symbols correspond to the following concentrations of FMPP: circles = no inhibitor, solid triangles = 20 μM, squares = 40 μM, +'s = 60 μM, ×'s = 80 μM, solid circles = 100 μM, solid squares = 200 μM, and triangles = 300 μM. (B) Hyperbolic plot of observed inactivation rate (k_{obs}) versus concentration of FMPP. (C) Double reciprocal form of the same plot. From the fit of the hyperbolic plot, the k_{inact} = 0.074 min^{-1} and K_I = 136 μM.

of TGT within 6 h. The inactivation was dependent upon the presence of tRNA (data not shown). The inactivated enzyme remained identical to native enzyme on both SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) and native PAGE gels and retained the ability to bind tRNA as shown by a previously described (Curnow & Garcia, 1994) native PAGE band shift assay (data not shown). Removal of unbound inhibitor from enzyme was accomplished by anion exchange chromatography. The repurified, inactivated enzyme exhibited no catalytic activity in the guanine exchange assay.

Both the time and concentration dependence of inactivation of TGT by FMPP were determined (Figure 3A). Due to the limited solubility of FMPP in aqueous media, the highest concentration of FMPP investigated was 300 μM. The

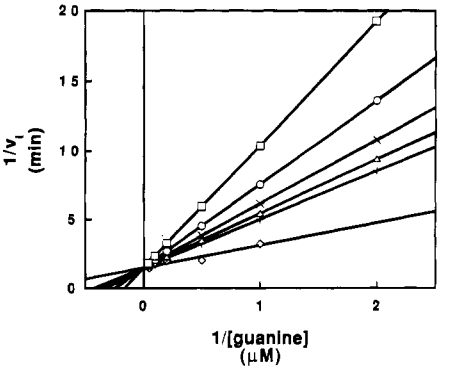

FIGURE 4: Competitive inhibition of TGT by FMPP under initial velocity conditions. Assay mixtures included 10–200 μM FMPP, 20 μM tRNA, 5–25 μM [8-^{14}C]guanine, and 1 μM TGT in HEPES assay buffer. Aliquots were taken at various times from 1 to 5 min and counted as described in Materials and Methods. These concentrations of TGT and guanine and the short time course were necessary to minimize curvature of the data due to irreversible inactivation. The symbols correspond to the following concentrations of FMPP: +'s = 10 μM, triangles = 25 μM, ×'s = 50 μM, circles = 100 μM, and squares = 200 μM.

results in Figure 3A clearly show a concentration dependence for inactivation of TGT by FMPP, with higher concentrations of inhibitor giving faster rates of inactivation. Inactivation of TGT by FMPP was also found to be linear with time. A hyperbolic fit of a replot of the observed rate of inactivation (k_{obs}) versus FMPP concentration (Figure 3B) gives a k_{inact} = 0.074 (±0.004) min^{-1} and K_I = 136 (±14) μM. A double reciprocal transformation of this replot is shown in Figure 3C.

Competitive Inhibition of TGT by FMPP. Higher concentrations of FMPP, lower concentrations of TGT or [8-^{14}C]-guanine, and shorter reaction times all resulted in significant curvature of the initial rate data, consistent with the occurrence of irreversible inactivation of the enzyme. A K_I of 114 (±1) μM for FMPP was determined with the results shown in Figure 4. The intersecting lines at the y-axis in the double reciprocal plot indicate a competitive mode of inhibition with respect to guanine.

Protection from Irreversible Inactivation of TGT by a Competitive Inhibitor. A competitive inhibitor of TGT, 7-methyl-preQ$_1$, was found to protect TGT from inactivation by FMPP (Figure 5). At a concentration of 7-methyl-preQ$_1$ approximately equal to its K_I (0.5 μM; Hoops *et al.*, 1995), ca. 50% reduction in the rate of inactivation (k_{obs}) of TGT by 100 μM FMPP is observed (k_{obs} = 0.018 min^{-1} versus 0.030 min^{-1} in the absence of the competitive inhibitor). At saturating concentrations of 7-methyl-preQ$_1$, the enzyme is completely protected against inactivation by 100 μM FMPP.

TGT-Catalyzed Elimination of Fluoride Ion from FMPP. The elimination of fluoride ion over time was observed for a mixture of 20 μM TGT, 300 μM FMPP, and 40 μM tRNA (Figure 6A). In the absence of tRNA substrate, the rate of fluoride release is drastically reduced. A control experiment in the absence of both TGT and tRNA is also shown in Figure 6A. Under these conditions, very low levels of fluoride ion (<10% of the total FMPP present) were detected, indicating that FMPP itself is stable under the incubation conditions. The initial velocity of fluoride release was determined to be 0.064 min^{-1} by computer fit to the linear portion of the time course for fluoride release (Figure 6B). For comparison, the inactivation time course for 300 μM

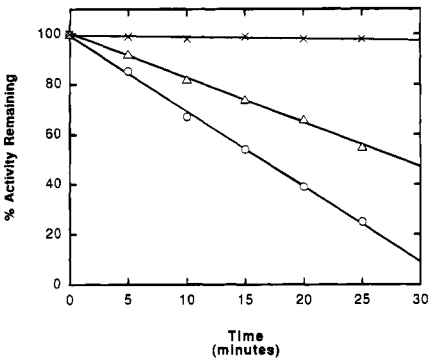

FIGURE 5: Protection of TGT against inactivation by FMPP by the competitive inhibitor 7-methyl-preQ$_1$. TGT (5 μM) was incubated at 37 °C with 100 μM FMPP and 7-methyl-preQ$_1$ at its K_I (0.5 μM) or at saturating concentration (20 μM) in HEPES assay buffer containing 20 μM tRNA. Aliquots were diluted 40-fold prior to determination of remaining activity as described in Materials and Methods. The symbols correspond to the following: circles = FMPP only, triangles = FMPP + 7-methyl-preQ$_1$ (0.5 μM), and $\times$'s = FMPP + 7-methyl-preQ$_1$ (20 μM).

FMPP is shown in Figure 6C. The k_{obs} for inactivation by 300 μM FMPP is 0.053 min^{-1}; however, it should be noted that the enzyme was present at 5 μM, and tRNA was 20 μM. The stoichiometry of fluoride release relative to TGT inactivation was estimated by fitting the fluoride release curve (Figure 6A) to a hyperbolic equation, giving an end point of fluoride release of 159 ($\pm$1.6) μM. This gives an 8:1 ratio of fluoride released to TGT inactivated.

Normal Turnover of FMPP by TGT. In order to determine the partition ratio for FMPP, the incorporation of FMPP into tRNA was determined utilizing an [8-^{14}C]guanine washout assay. The hyperbolic plot of initial velocity versus FMPP concentration is shown in Figure 7. Nonlinear fit of the Michaelis–Menten equation gives a K_m of 152 ($\pm$1) μM and a k_{cat} of 0.461 ($\pm$0.001) min^{-1}.

DISCUSSION

In *E. coli*, tRNA-guanine transglycosylase catalyzes the base exchange of G34 in queuine-cognate tRNAs (Asn, Asp, His, and Tyr) with the queuine precursor, preQ$_1$. This reaction involves the cleavage of the G34 glycosidic bond and the formation of a new glycosidic bond to the N-7 of preQ$_1$. The preQ$_1$ base is further modified to queuine by two subsequent reactions after incorporation into tRNA. While the chemical mechanism for the transglycosylation reaction is unknown, inspection of the substrates and products indicates that N-7 of preQ$_1$ must be deprotonated in order to form a new glycosidic bond. With this in mind, we designed a potential mechanism-based inactivator of TGT by substitution of a fluoromethyl group for the aminomethyl group of preQ$_1$. Upon deprotonation by TGT, this analogue, FMPP, should be able to eliminate fluoride ion, forming a Michael acceptor in the enzyme active site (Figure 8). A suitably positioned enzymic nucleophile could then attack the Michael acceptor, yielding a covalently inactivated TGT.

Preliminary experiments indicated that FMPP does indeed inactivate TGT and is dependent upon the presence of tRNA, consistent with a mechanism-based inactivation. Preincubation studies indicate that FMPP inhibits TGT in a concentration- and time-dependent manner (Figure 3A). Fitting a replot of k_{obs} versus FMPP concentration (Figure 3B) to the Michaelis–Menten equation reveals that the

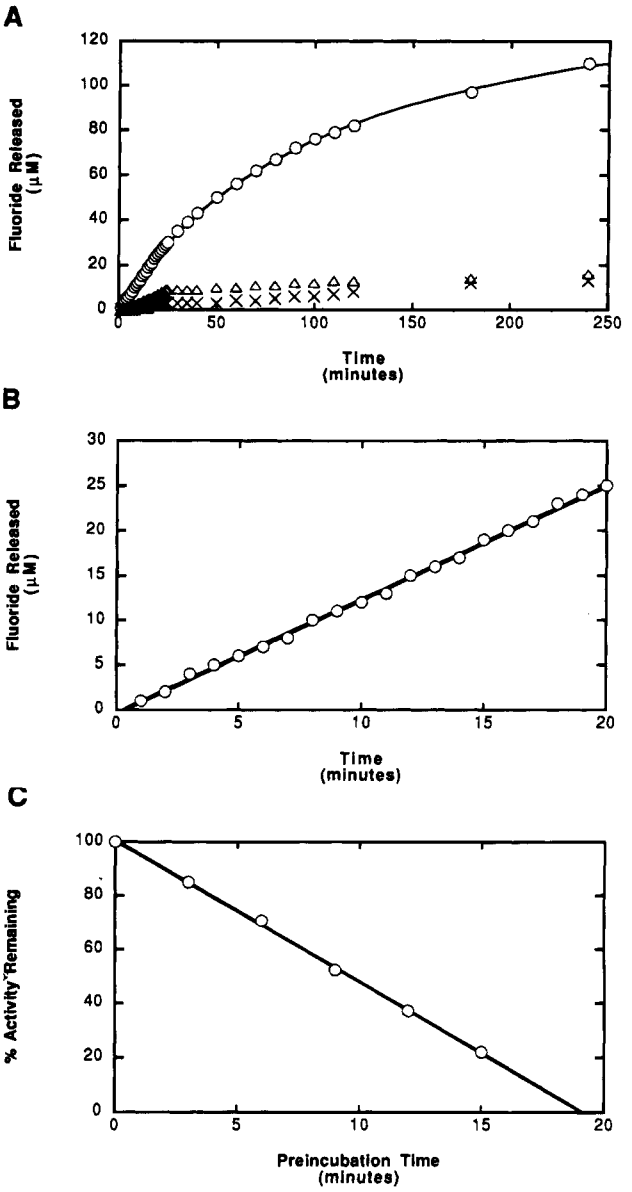

FIGURE 6: TGT-catalyzed fluoride release from FMPP. (A) Fluoride concentration was determined using a fluoride ion-selective electrode as described in Materials and Methods. The reaction solution contained 300 μM FMPP, 20 μM TGT, and 40 μM tRNA in HEPES assay buffer. The symbols correspond to the following: circles = FMPP + TGT + tRNA, triangles = FMPP + TGT − tRNA, and $\times$'s = FMPP − TGT − tRNA. (B) Fluoride release from FMPP (300 μM) in the presence of TGT (20 μM) and tRNA (40 μM). (C) Inactivation of TGT (5 μM) in the presence of 300 μM FMPP and 20 μM tRNA.

inactivation is a saturable process with k_{inact} = 0.074 min^{-1} and K_I = 136 μM. Separation of the inactivated TGT from unbound FMPP by anion exchange chromatography did not result in a regeneration of enzyme activity, indicating that FMPP is either covalently attached or at least very tightly bound to TGT.

TGT catalyzes the exchange of free guanine for G34 in tRNA. This provides a convenient assay for TGT activity where the incorporation of radiolabel from [8-^{14}C]guanine into tRNA is monitored. We have found that the kinetic parameters for guanine incorporation are similar to those for preQ$_1$ incorporation (Hoops et al., 1995); therefore, the guanine exchange reaction is a reasonable measure of TGT activity. Our results indicate that FMPP acts as a competitive

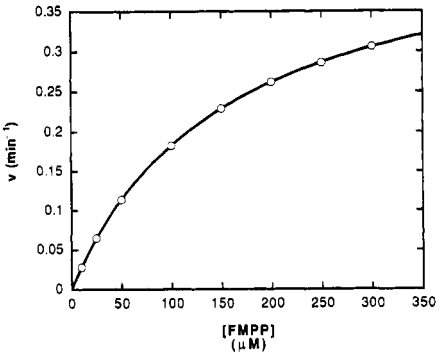

FIGURE 7: Normal turnover of FMPP by TGT. Hyperbolic plot of initial velocity versus FMPP concentration determined by monitoring the loss of radioactivity in prelabeled G*-tRNA as described in Materials and Methods.

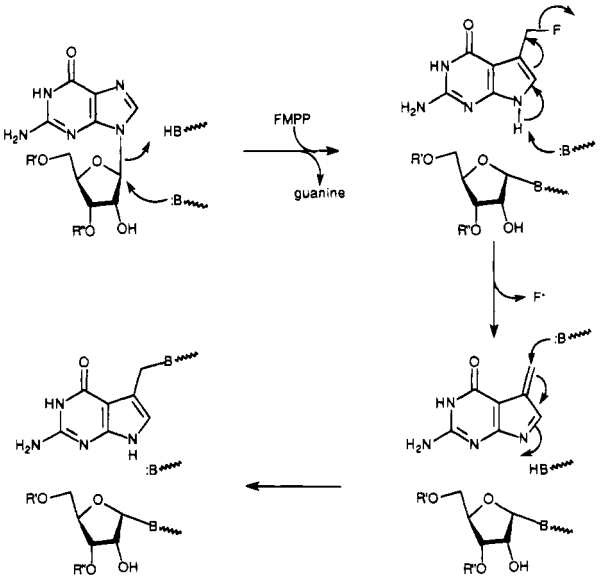

FIGURE 8: Proposed mechanism for inactivation of TGT by FMPP.

inhibitor of TGT with respect to guanine under initial velocity conditions (Figure 4) with a $K_I = 114\ \mu M$, in good agreement with the K_I of 136 μM determined for inactivation. Relatively high concentrations of guanine and TGT and shorter time courses were required to determine the competitive inhibition by FMPP in order to avoid curvature of incorporation versus time plots due to inactivation of the enzyme. This is a common feature of mechanism-based inactivators (Santi & Kenyon, 1980).

We have previously characterized 7-methyl-preQ$_1$ as a competitive inhibitor, with respect to guanine, of the TGT reaction with a K_I of 0.5 μM (Hoops *et al.*, 1995). 7-Methyl-preQ$_1$ protects TGT from inactivation by FMPP. As shown in Figure 5, this protection is concentration-dependent with ca. 50% protection at a concentration of 7-methyl-preQ$_1$ equal to its K_I and essentially complete protection at a saturating concentration of 7-methyl-preQ$_1$.

Taken together, these data indicate that FMPP is an active site-directed, irreversible inhibitor of TGT and suggest that the inhibition is mechanism-based. In order to further investigate the mode of FMPP inhibition of TGT, we have monitored the release of fluoride ion from FMPP. The fluoride ion release was dependent upon the presence of both TGT and tRNA (Figure 6A). The initial rate of fluoride release was slightly faster (0.064 min^{-1}) than the observed inactivation rate (0.053 min^{-1}) at the same concentration of

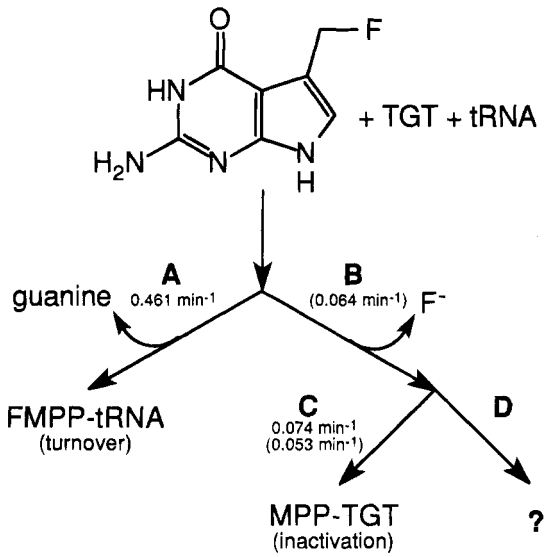

FIGURE 9: Scheme for the TGT-catalyzed processing of FMPP. Route A corresponds to the normal turnover of the enzyme. Route C corresponds to irreversible inactivation of TGT following fluoride release (B). Route D corresponds to an alternative processing of FMPP by TGT involving fluoride release (B), yielding an unknown product and no inactivation of the enzyme. The rates for the turnover, fluoride release, and inactivation are shown. The rates in parentheses (fluoride release and inactivation) correspond to 300 μM FMPP due to limited solubility of the compound.

FMPP, consistent with fluoride release preceding inactivation in a mechanism-based mode of inactivation.

FMPP was also investigated as a normal substrate for TGT; that is, the kinetics of incorporation of FMPP into tRNA were determined. A guanine washout assay was used to determine that FMPP is incorporated into tRNA with a K_m of 152 μM and a k_{cat} of 0.461 min^{-1}. This K_m is in reasonable agreement with the K_I's determined for inactivation and for competitive inhibition, suggesting that a similar (if not identical) Michaelis complex is formed in each case.

A scheme for the partitioning of FMPP is shown in Figure 9. The rates for the turnover (A), fluoride release (B), and inactivation (C) are shown. The rates in parentheses (fluoride release and inactivation) correspond to 300 μM FMPP due to limited solubility of the compound. The rate of incorporation of FMPP compared to the inactivation rate indicates a partitioning of 1:8 of inactivation to turnover. The stoichiometry of fluoride release (ca. 160 μM versus 20 μM TGT) gives an 8:1 ratio of fluoride released to TGT inactivated. This suggests that TGT is processing FMPP to an alternative product that does not involve inactivation of the enzyme. It may be that fluoride can be released from FMPP−tRNA. An analysis of the products of the inactivation incubation will be necessary for characterization of this alternative product.

A study of a series of 5-substituted 2-aminopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (Hoops *et al.*, 1995) suggests that the role of the aminomethyl substituent of preQ$_1$ is primarily, if not solely, one of recognition and binding. It is therefore reasonable to expect that the enzyme will use one or more residues to bind the aminomethyl functional group. If these residues are acting as hydrogen bond acceptors, then they may be nucleophilic enough to attack the Michael acceptor formed from deprotonation and fluoride release from FMPP. The 2-amino-(5-chloromethyl-, 5-bromomethyl-, and 5-iodomethyl)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones have been syn-

thesized. Preliminary experiments indicate that each of these compounds inactivates TGT. Further experiments to characterize the inactivation of TGT by these compounds and to identify the enzymic residue (or residues) that are alkylated are in process.

Our results are consistent with FMPP acting as a mechanism-based inactivator of tRNA-guanine transglycosylase from *E. coli*. This confirms our earlier observation (Hoops *et al.*, 1995) that the acidity/nucleophilicity of N-7 of the pyrrolopyrimidine substrate for TGT is important in the TGT reaction. This inhibitor (FMPP), if it possesses suitable physical properties for cellular uptake, may serve as a useful biological probe for the role of the queuine modification in tRNA as well as an *in vitro* mechanistic probe for the *E. coli* TGT.

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